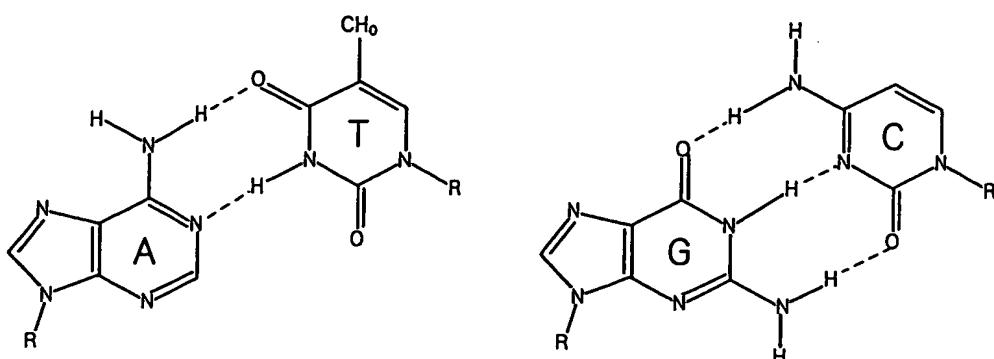
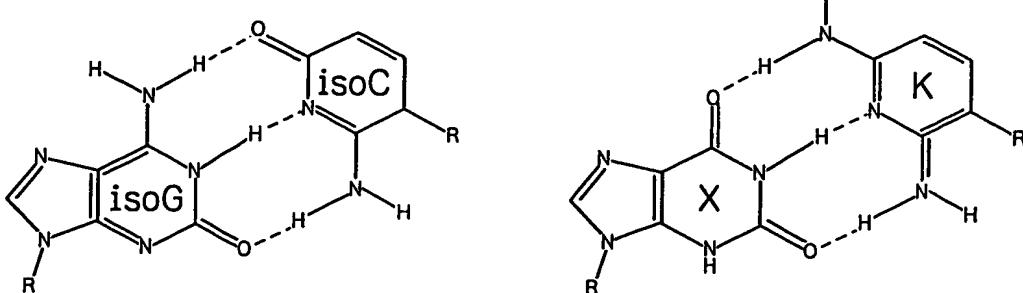


Figure 1

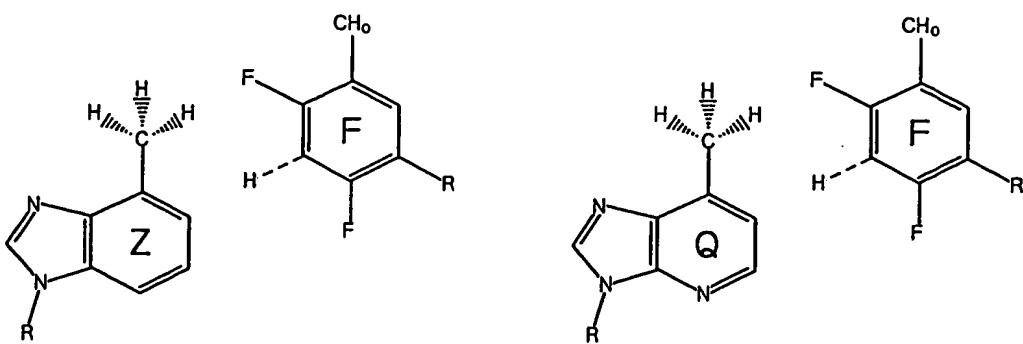
a)



b)



c)



d)

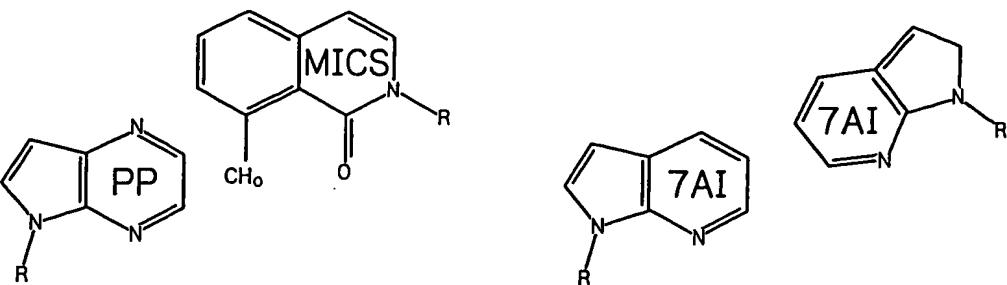


Figure 2

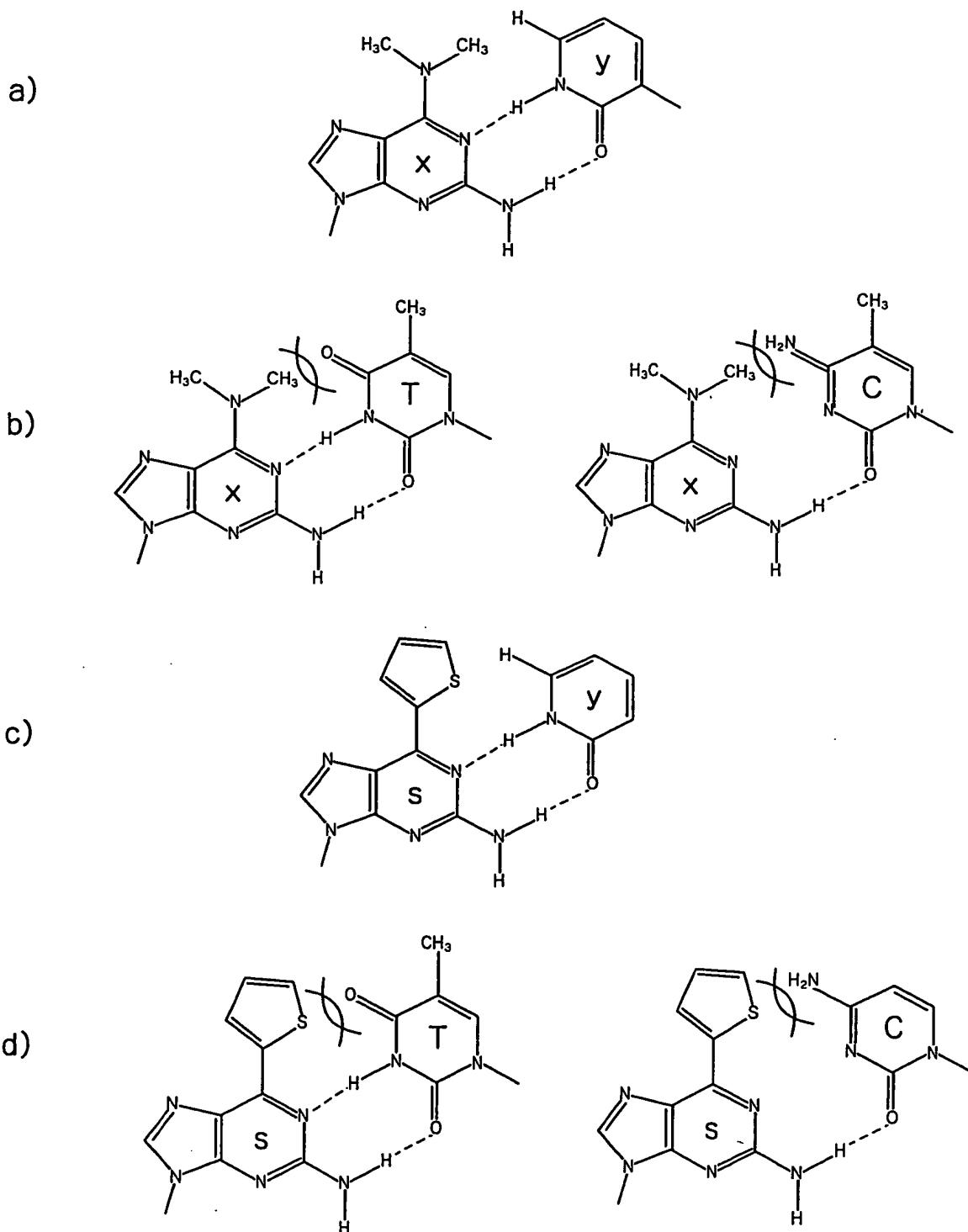
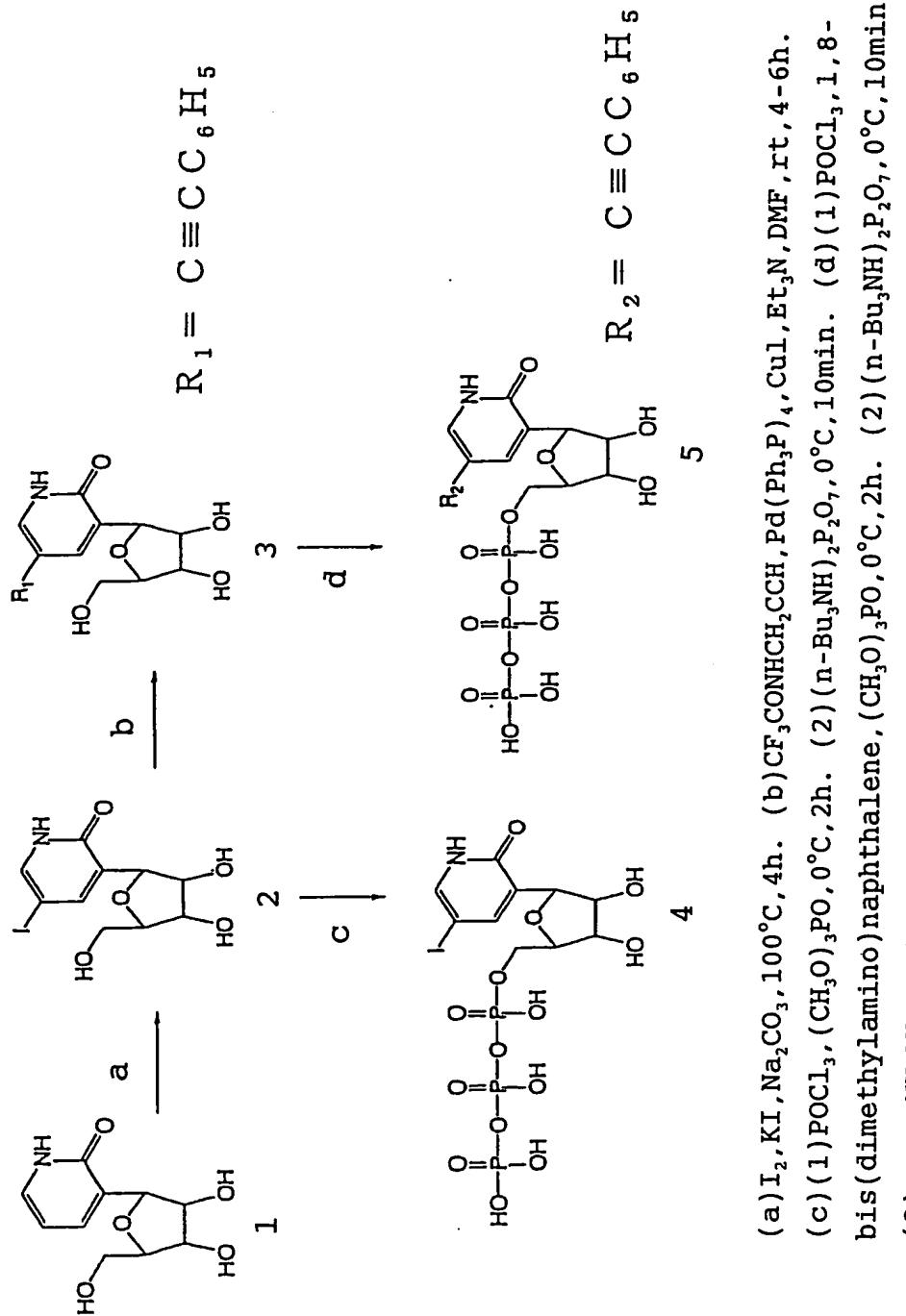


Figure 3



A

App No.: 10/521,454

Docket No.: 0230-0222PUS1

Inventor: Ichiro HIRAO et al.

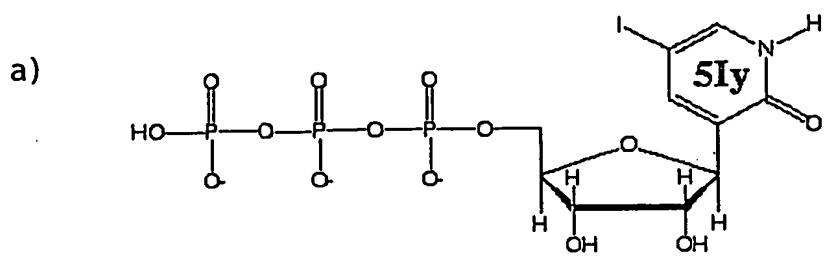
Title: NUCLEOSIDE OR NUCLEOTIDES HAVING NOVEL

UNNATURAL BASES AND USE THEREOF

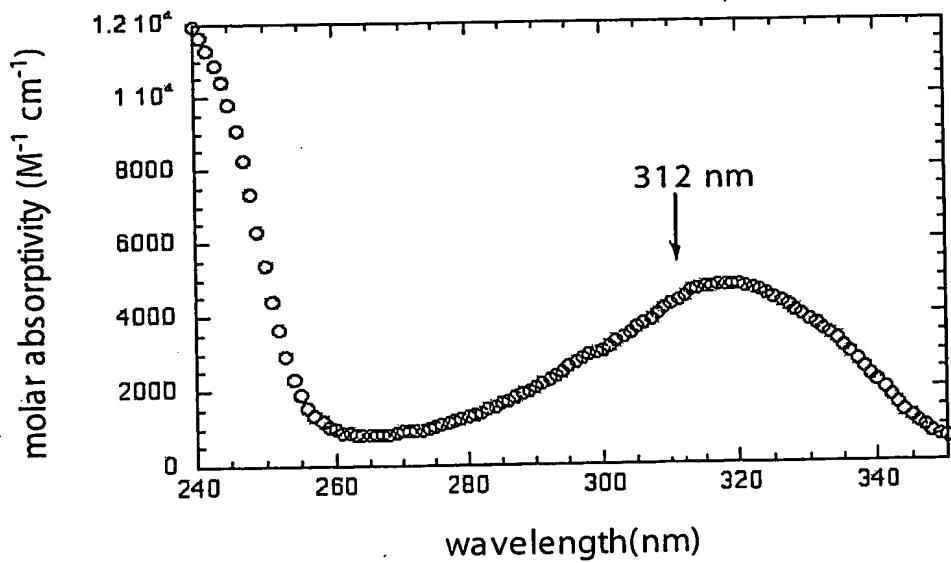
REPLACEMENT SHEET

Sheet 4 of 20

Figure 4



b)



## Figure 5

a)

5'-end primer; 39-mer

39.45 : 5' -GGTAATACGACTCACTATAGGGAGTGGAGGAATTCATCG

3'-end primer; 29-mer

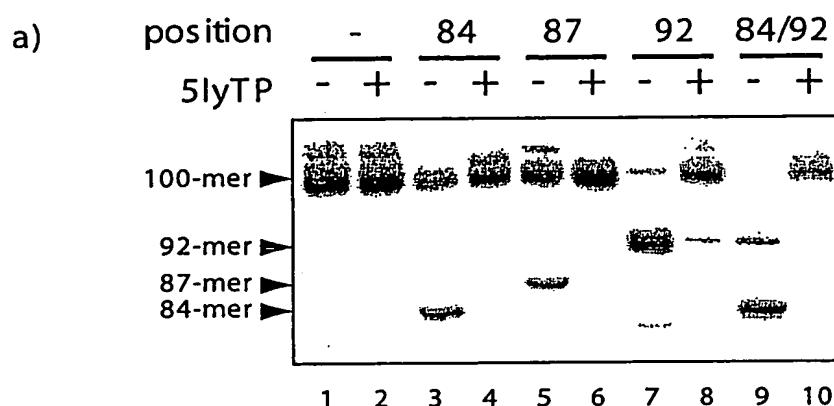
29.45 : 5' -GCAGAAGCTTGTGCTAAGGCATATG

29.45s84 : 5' -GCAGAAGCTTGTGCTsCTAAGGCATATG29.45s87 : 5' -GCAGAAGCTTGTsCGCTAAGGCATATG29.45s92 : 5' -GCAGAAGCsTGCTGTGCTAAGGCATATG29.45s84/92 : 5' -GCAGAAGCsTGCTGTsCTAAGGCATATG

b)

5' - GGGAGUGGGAG GAAUUCAUCG AGGCAUAUGU CGACUCCGUC UUCCUUCAA  
CCAGUUUAUA AUUGGUUUUA GCAUAUGCCU UAGCGACAGC AAGCUUCUGC

Figure 6



b)

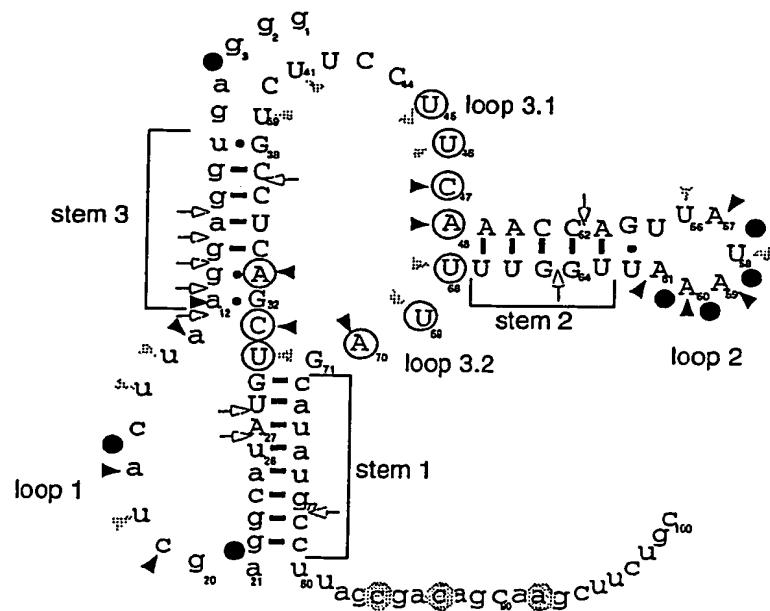


Figure 7

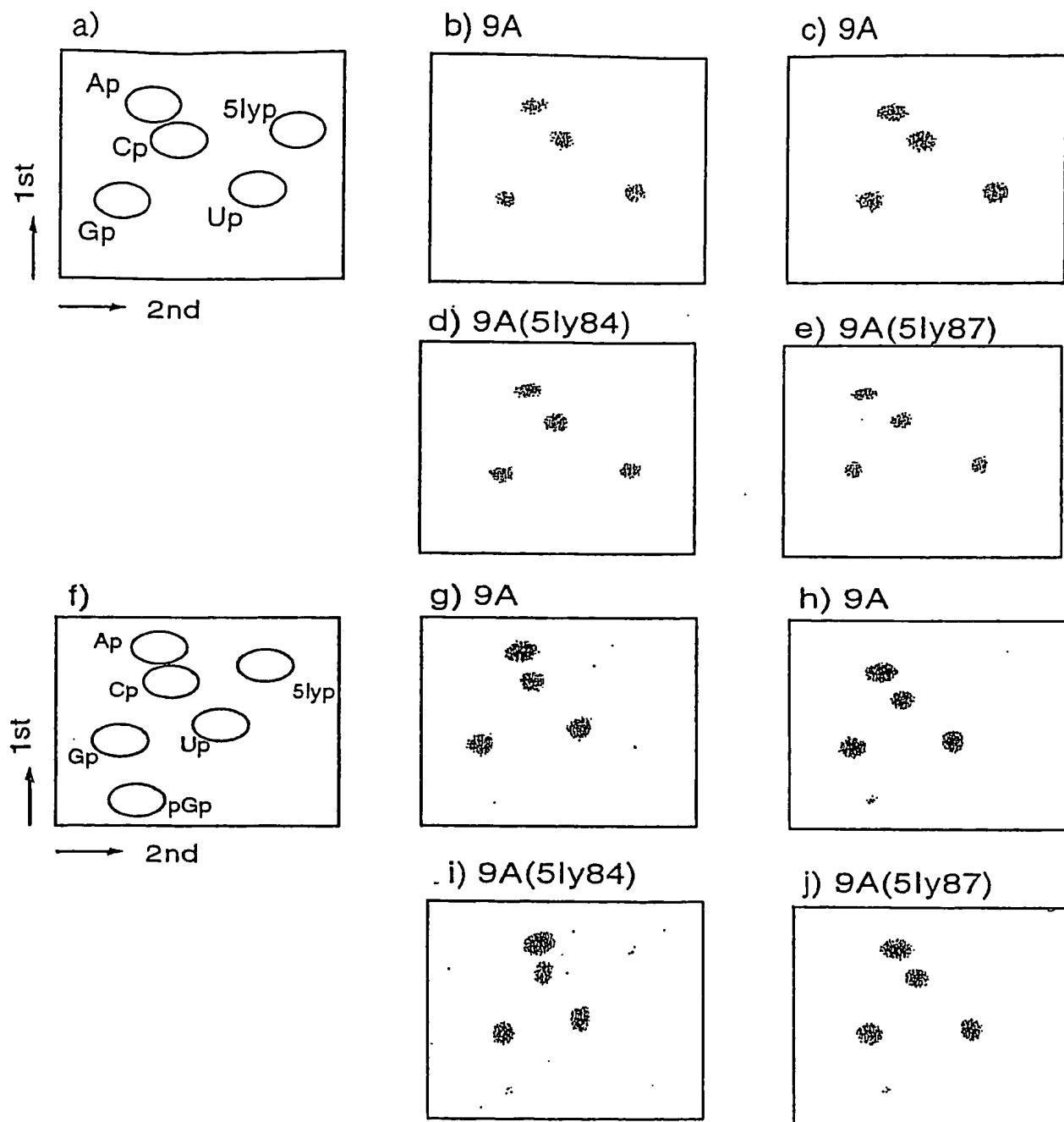


Figure 8

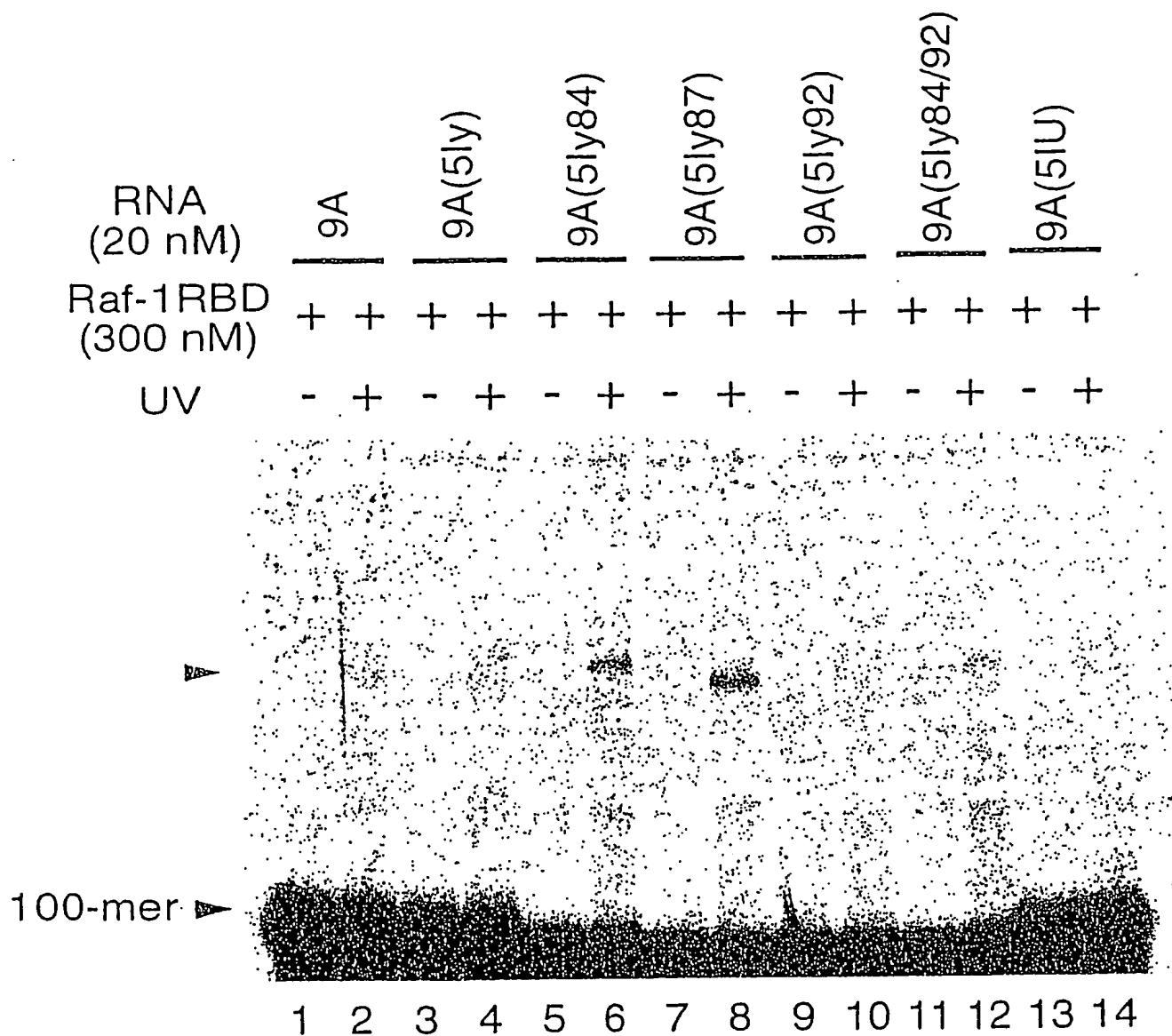


Figure 9

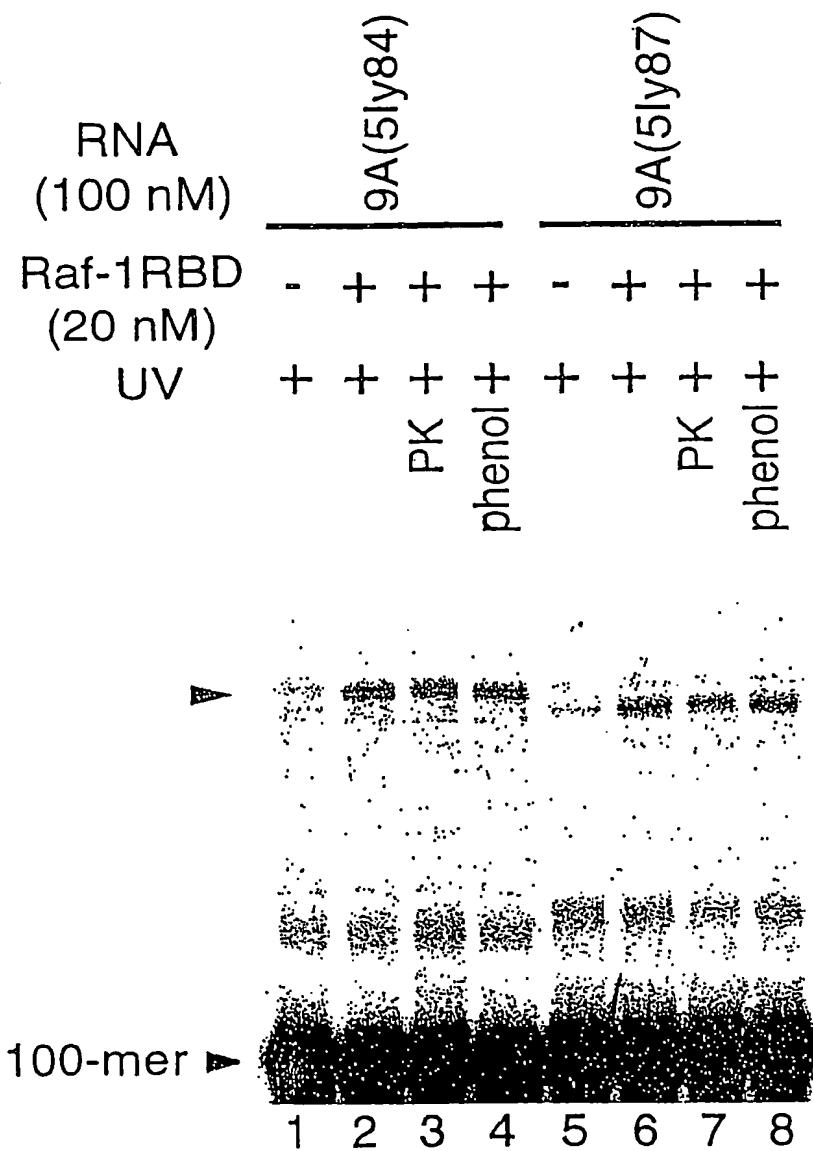
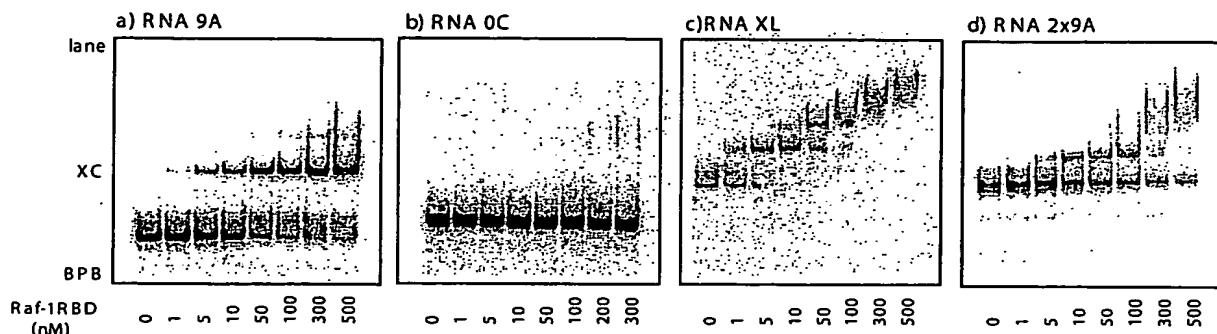


Figure 10



## e) RNA 9A :100-mer

5' -GGGAGUGGAGGAAUUCAUUCGAGGCAU [-N<sub>45</sub>-] CAUAUGCCUUAGCGACAGCAAGCUUCUGC-3'  
 AUGUCGACUCGUCUUCCUAAACCAGUUAAAUGGUUUAG

## RNA 9A(5ly87) :100-mer

5' -GGGAGUGGAGGAAUUCAUUCGAGGCAU [-N<sub>45</sub>-] cauaugccuuagcga5lyCAGCAAGCUUCUGC-3'

## RNA 2x9A :200-mer

5' -GGGAGUGGAGGAAUUCAUUCGAGGCAU [-N<sub>45</sub>-] CAUAUGCCUUAGCGACAGCAAGCUUCUGC-  
 -GGGAGUGGAGGAAUUCAUUCGAGGCAU [-N<sub>45</sub>-] CAUAUGCCUUAGCGACAGCAAGCUUCUGC-3'

## RNA 0C :100-mer

5' -GGGAGUGGAGGAAUUCAUUCGAGGCAU [-N<sub>45</sub>-] CAUAUGCCUUAGCGACAGCAAGCUUCUGC-3'  
 CUGGGAAACCCUAUCUUGCUUUUGGUAGCUGUAUUCACCUACAG

RNA XL : cross-linking product generated from two molecules of 9A(5ly87)

Figure 11

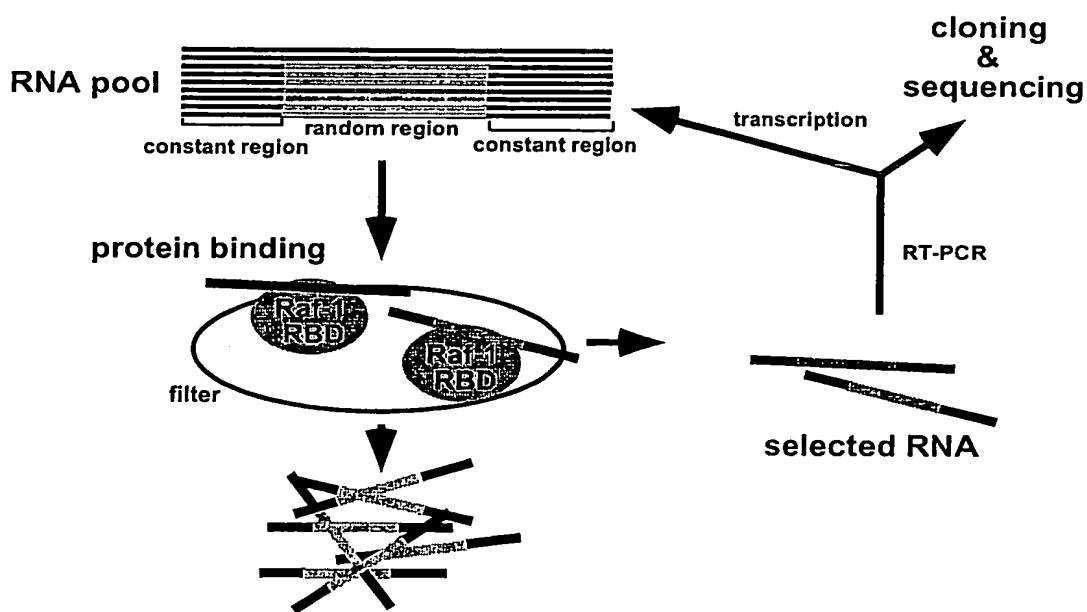


Figure 12

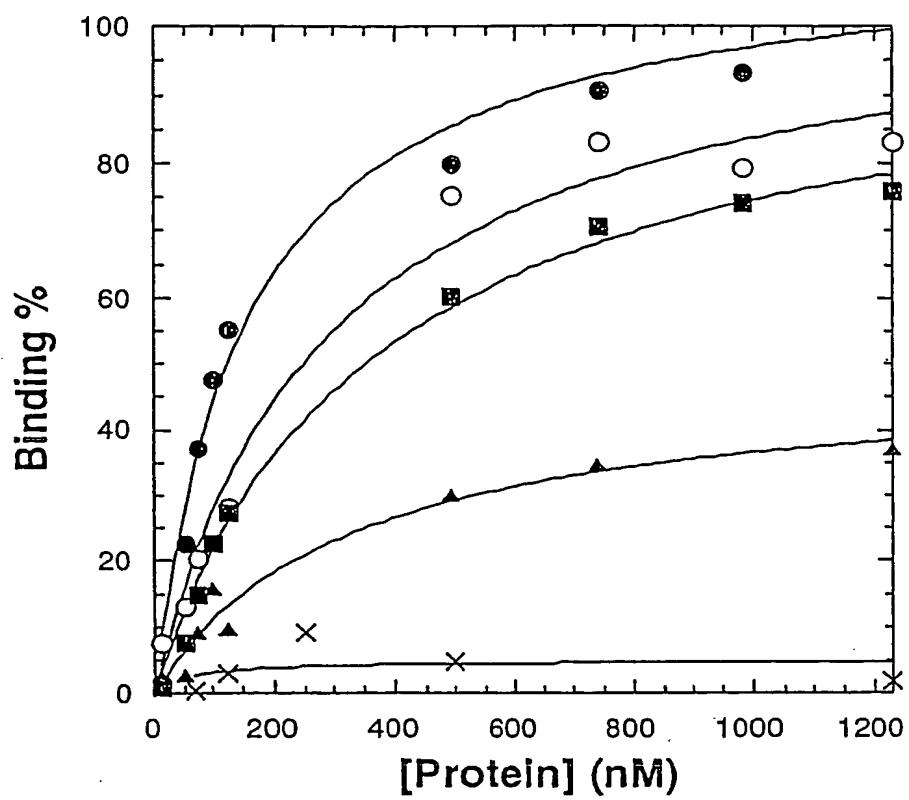
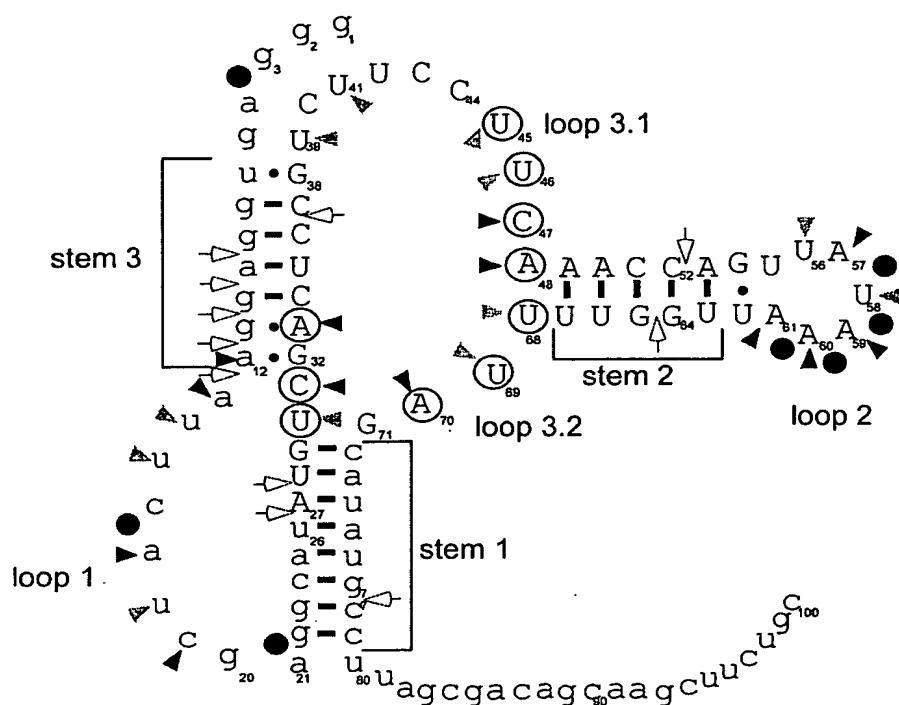


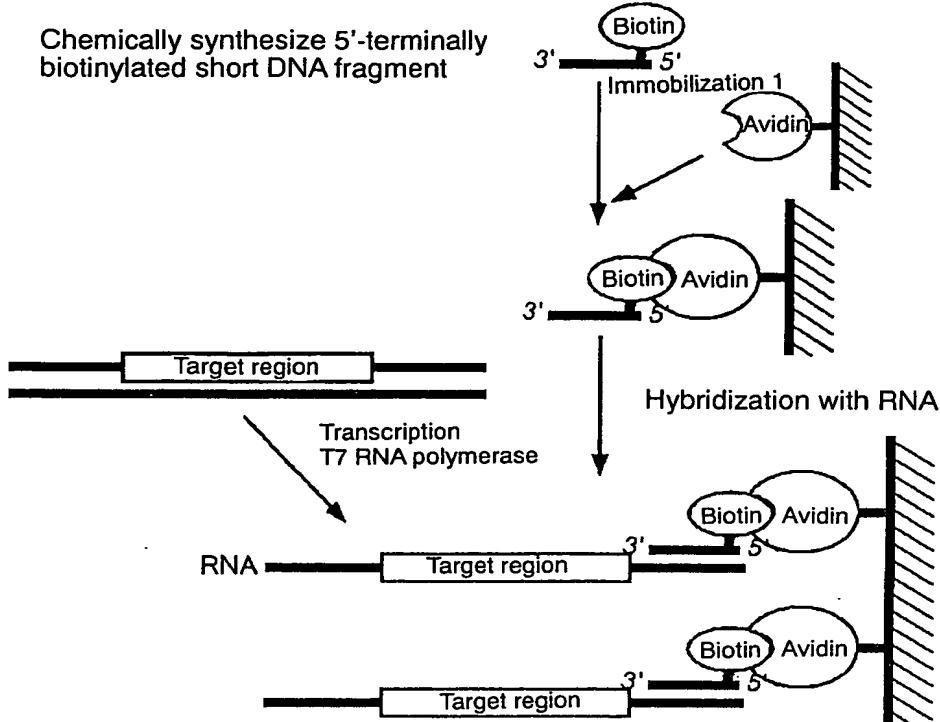
Figure 13



- ← RNase V1 cleavage
- Mung Bean Nuclease cleavage
- ▲ CMCT modification
- ◀ DMS modification
- (N) protection from chemical modification by the Raf-1 GST-RBD

Figure 14

## Conventional Method 1



## Conventional Method 2

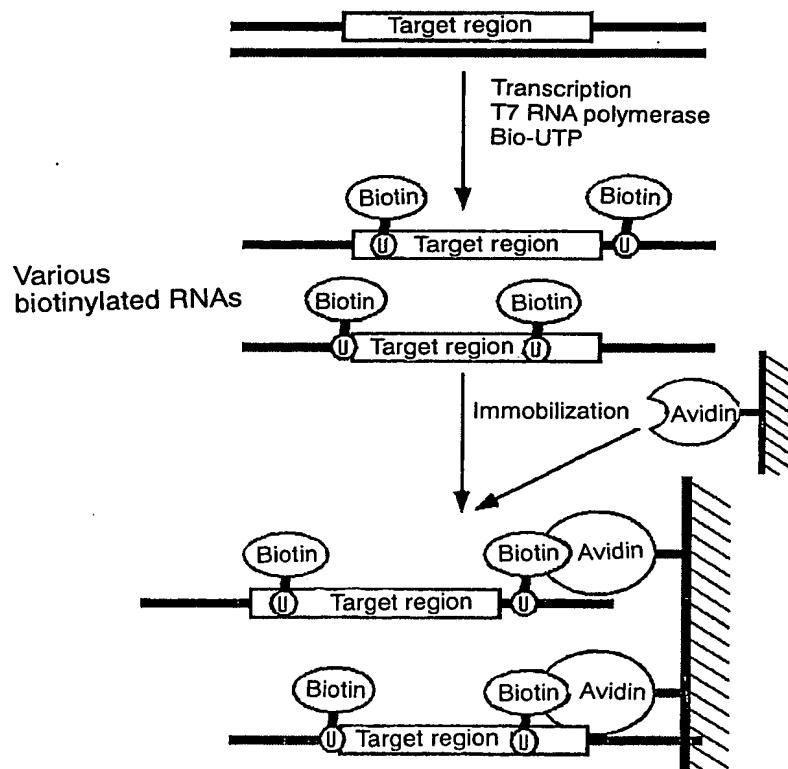
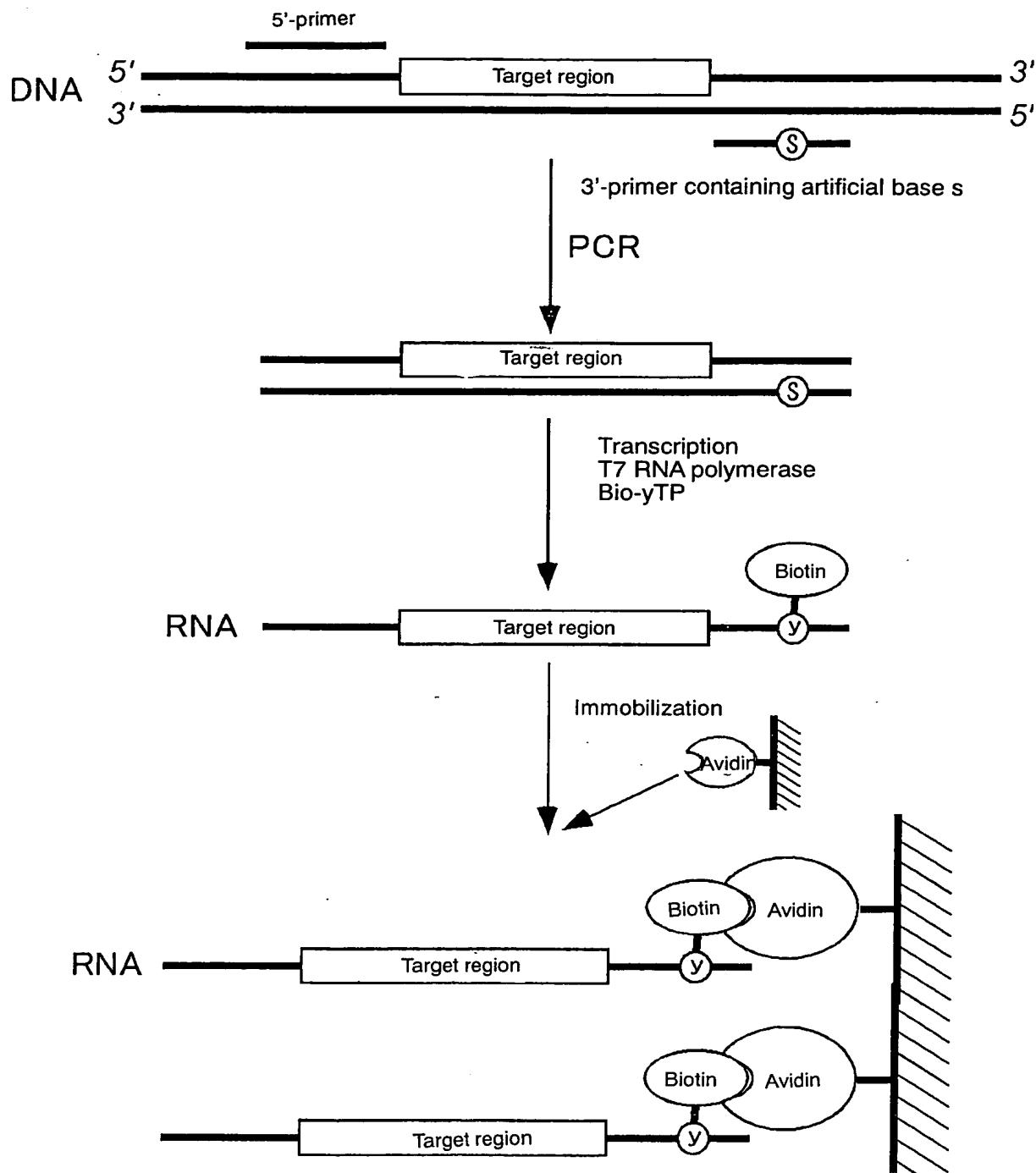


Figure 14 (Continued)

## Inventive Method based on artificial base pairing



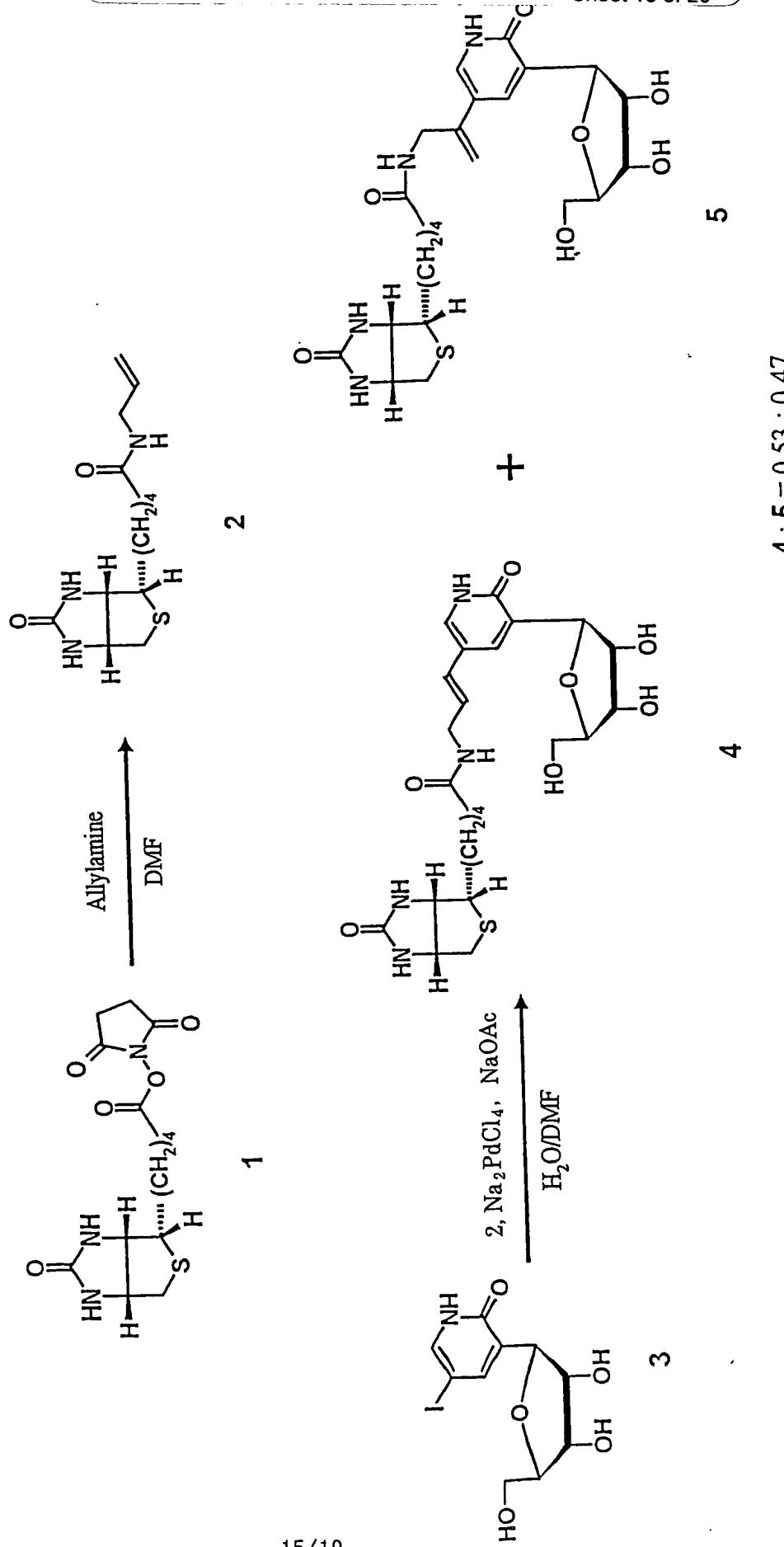


Figure 15

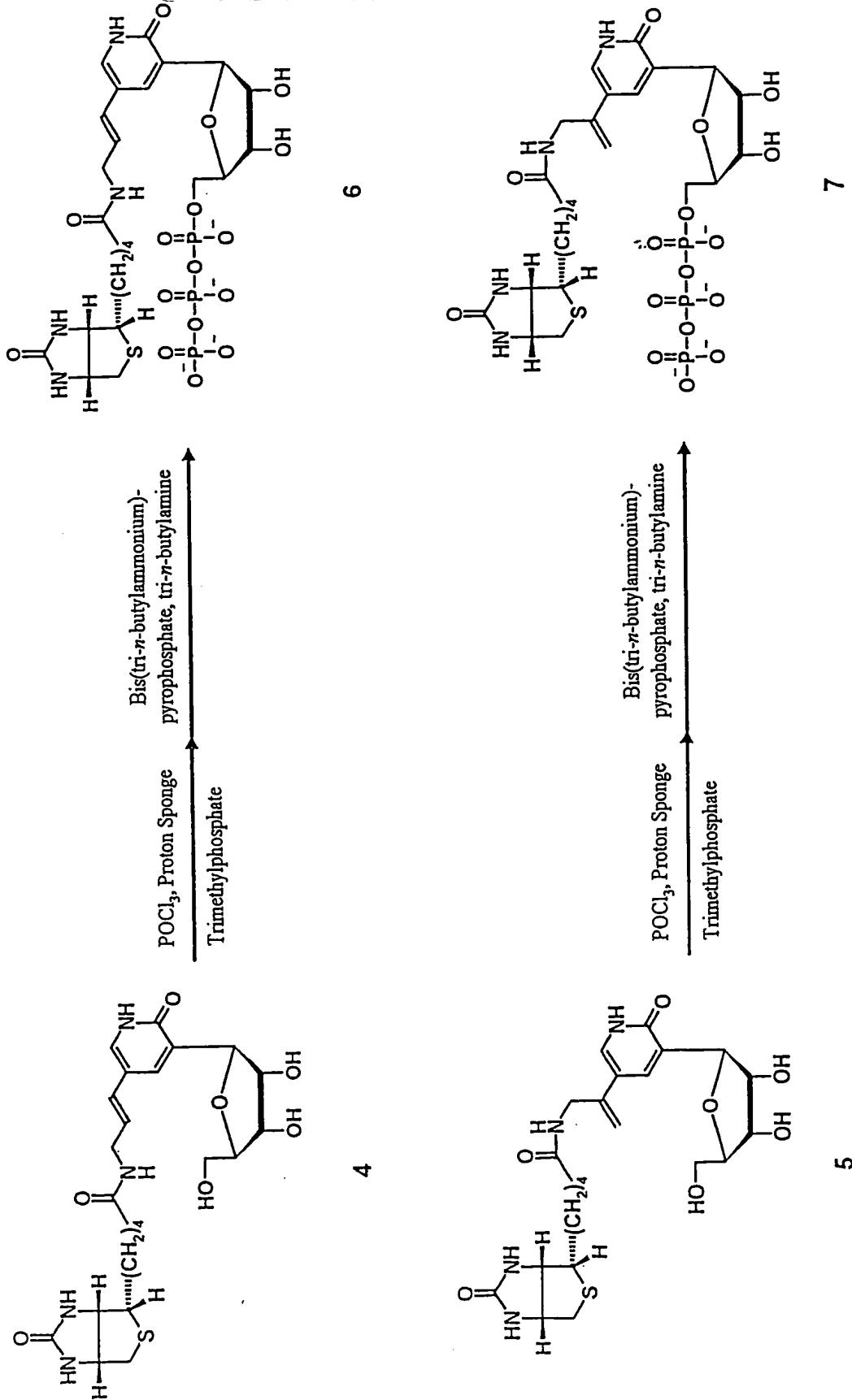


Figure 16

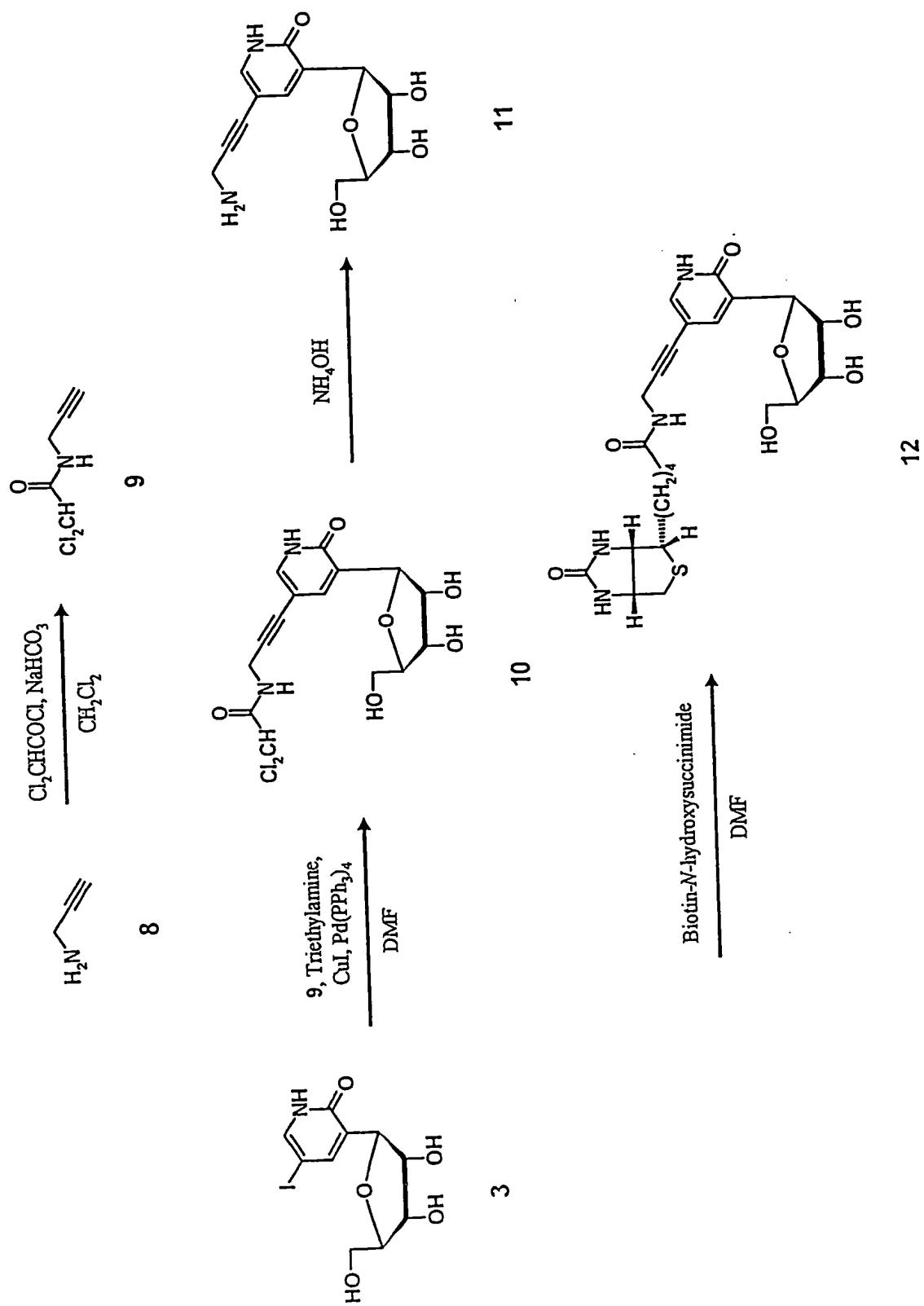


Figure 17

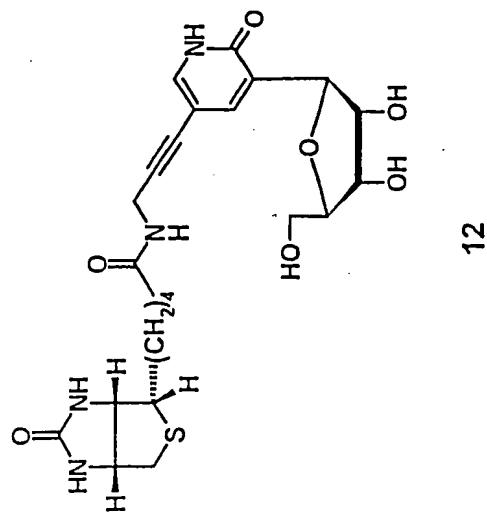
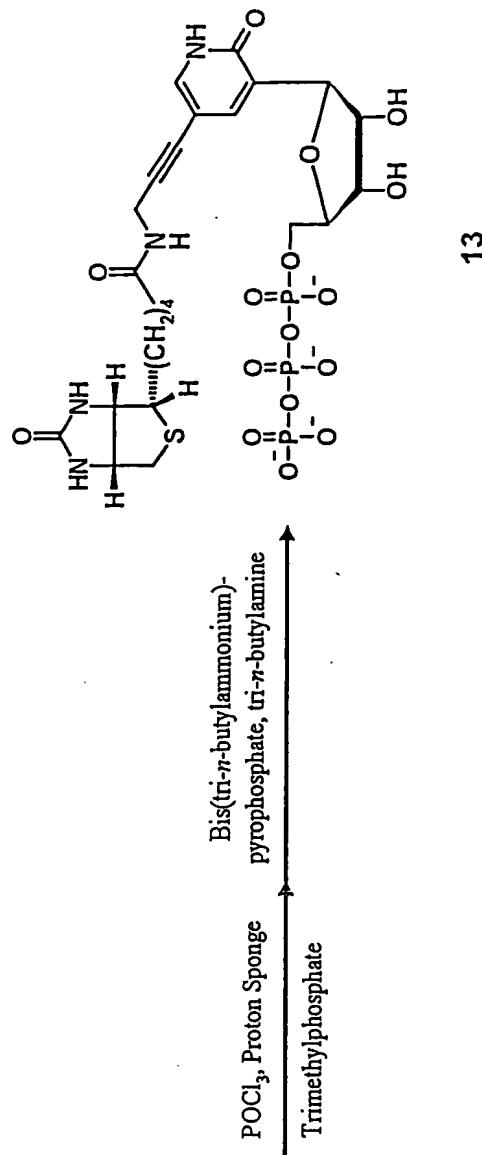


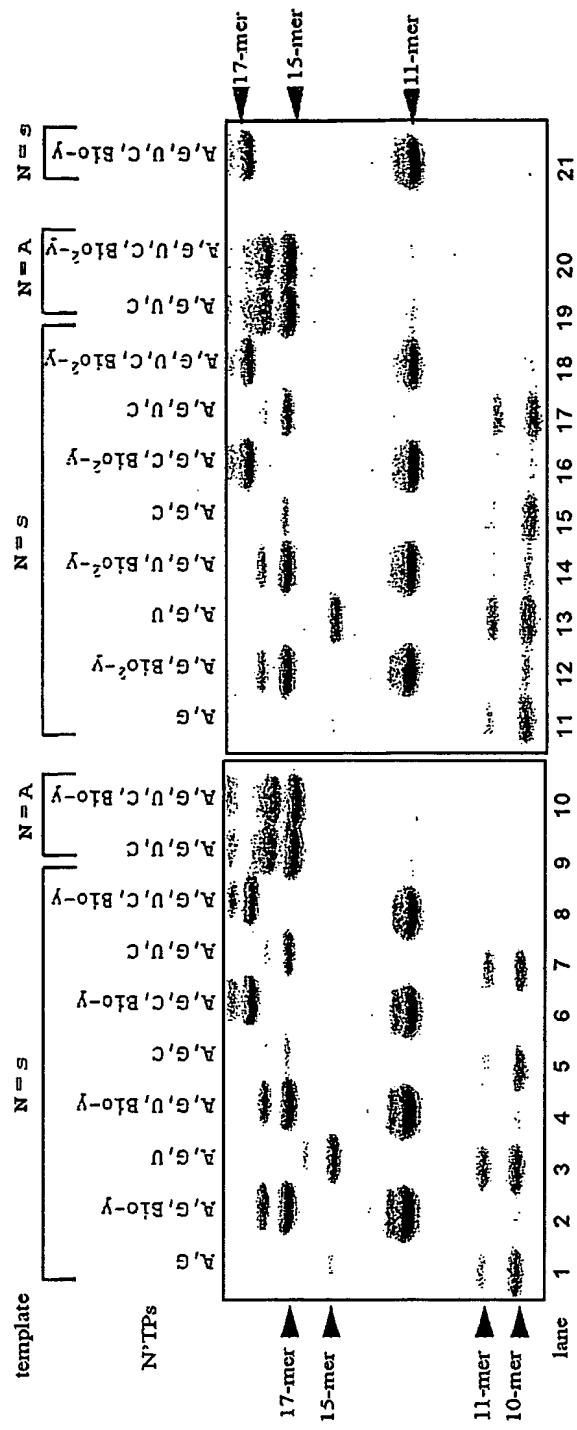
Figure 18

non-template strand (T7 prim 28N): 5'-d(ATAATCGACTCTACTATAGGGAGAAGA)  
 template strand (temp p35s or temp p35A): 3'-d(TATTATGCTGAGTGATATCCCTCTCTCTCGT) N = s or A  
 +1      +15      +17

T7 RNA polymerase

↓ 1 mM N'NTPs, [ $\alpha$ -32P]ATP, 10 mM GMP

in the absence of CTP: 15-mer RNA (GGGAGGAAGAN' AGAG)  
 in the presence of CTP: 17-mer RNA (GGGAGGAAGAN' AGAGCA)



► RNA containing only normal nucleotides

► RNA containing normal nucleotides and Bio-Y or Bio2'-Y

Figure 19